Animal Reproduction

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Postpartum uterine health of superprecocious primiparous *Bos indicus* cows

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Fertility and uterine health during the postpartum period are crucial for achieving adequate reproductive performance in beef herds. However, superprecocious primiparous cows (PSP) present distinct characteristics such as sexual precocity and early body maturity, which influence their productive efficiency. This study aimed to evaluate postpartum uterine inflammation and fertility in superprecocious primiparous *Bos indicus* beef cows subjected to timed artificial insemination (TAI) protocols. Nelore PSP cows (*Bos indicus*, n = 268) were subjected to a conventional Estradiol-progesterone (E2-P4) based TAI protocol (D0, 2 mg of E2 benzoate and insertion of P4 intravaginal device; D8, removal of intravaginal P4 device, 150 µg of D-Cloprostenol PGF, 300 IU of eCG, and 1 mg of E2 cypionate; Day 10, TAI). Pregnancy diagnosis was performed by transrectal ultrasonography 30 days after TAI. The experimental groups were determined according to days postpartum (DPP) at the beginning of TAI program. For that purpose, optimal cut-off points for determining the relationship between the DPP and pregnancy status at 1st TAI were calculated using the receiver operating characteristic (ROC) curve. Therefore, cows were divided, as follows: 1) Early PSP (n = 59), cows between 25 and 31 DPP; 2) Late PSP (n = 209), cows between 32 and 60 DPP. On Day 0, before the start of the protocol, cows underwent cytological collection of uterine tissue using the cytobrush technique. The samples collected from uterine tissue were fixed on glass slides, stained with a Panotic kit, and subjected to a 200-cell counting under an optical microscope. After cytology sampling, the cytological brushes were individually stored in microtubes with RNAlater®. Total RNA was extracted from a subgroup of the samples (26 and 32 from Early PSP and late PSP, respectively), and gene expression analysis for IL-1β, IL-8, TNF, PGR, ESR1, ESR2, and PTCH2 was performed by quantitative real-time PCR (qPCR-RT). The proportion of PMN and gene expressions were analyzed by analysis of variance (ANOVA), and the means were compared between groups using Tukey's test. Pregnancy per AI (P/AI) was analyzed by Chi-square test. The P/AI was lower in Early PSP cows than in Late PSP cows ($P < 0.01$; 45.7%, 27/59, and 66%, 138/209, respectively). Early PSP cows had a higher proportion of PMN cells than Late PSP cows (22,1% and 5,2%, respectively; $P < 0.001$). The gene expression of IL-1β, IL-8, TNF, ESR1, ESR2, and PTCH2 was higher ($P < 0.05$) in Late PSP cows than in Early PSP cows. The PGR gene expression tended to be lower ($P = 0.08$) in Early PSP cows than in Late PSP cows. These results show that PSP cows subjected to early TAI protocols (< 31 DPP) have a higher proportion of PMN and lower P/AI than late IATF (≥ 32 DPP). Intriguingly, in a uterine inflammatory and receptivity perspective, cows from the Late group had higher expressions of pro- inflammatory cytokine genes (IL-1β, IL-8, and TNF), and of uterine receptivity genes (ESR1, ESR2, and PTCH2).